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A second lineage of mammalian major histocompatibility complex class I genes

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ABSTRACT **Major histocompatibility complex (MHC) class I genes typically encode polymorphic peptide-binding chains which are ubiquitously expressed and mediate the recognition of intracellular antigens by cytotoxic T cells. They constitute diverse gene families in different species and include the numerous so-called nonclassical genes in the mouse *H-2* complex, of which some have been adapted to variously modified functions. We have identified a distinct family of five related sequences in the human MHC which are distantly homologous to class I chains. These *MIC* genes (*M*HC class *I* chain-related genes) evolved in parallel with the human class I genes and with those of most if not all mammalian orders. The *MICA* gene in this family is located near *HLA-B* and is by far the most divergent mammalian MHC class I gene known. It is further distinguished by its unusual exon–intron organization and preferential expression in fibroblasts and epithelial cells. However, the presence of diagnostic residues in the MICA amino acid sequence translated from cDNA suggests that the putative MICA chain folds similarly to typical class I chains and may have the capacity to bind peptide or other short ligands. These results define a second lineage of evolutionarily conserved MHC class I genes. This implies that *MICA* and possibly other members in this family have been selected for specialized functions that are either ancient or derived from those of typical MHC class I genes, in analogy to some of the nonclassical mouse *H-2* genes.**

Typical class I genes in the major histocompatibility complex (MHC) of all vertebrate species encode membrane-anchored cell surface glycoproteins of about 44 kDa that are noncovalently associated with β_2-microglobulin (1, 2). These molecules are receptors for intracellular peptides, which they present to cytotoxic T cells with $\alpha\beta$ antigen receptors, thus facilitating the immune recognition of intracellular pathogens and altered self-proteins. Characteristic of typical MHC class I chains is their polymorphism, which correlates with the ability of various alleles to bind peptides with different sequence motifs (3). At least to some extent, the allelic repertoire of a typical class I chain represents an evolutionary record of past pathogen-driven selection (4). This principle may also be accountable for the diversity of MHC class I genes among different species and, in several cases, for their adaptation to specialized functions. But such examples have so far only been found in the mouse. In addition to the typical class I *H-2K*, *-D*, and *-L* genes, the mouse *H-2* complex includes numerous of the nonclassical *Q*, *T*, and *M* genes (5–8). Of these, some of the *T* genes encode ligands for T cells with $\gamma\delta$ antigen receptors (9, 10) and the *M3* gene product specifically presents N-formylated peptides of bacterial and mitochondrial origin (11, 12).

The human MHC contains within about 2 megabases six class I genes (13), of which *HLA-A*, *-B*, and *-C* encode the polymorphic antigen-presenting molecules (2). The physiological roles of HLA-E and -F are uncertain (14, 15), but HLA-G may have a specific function at the maternal–fetal interface (16). In addition, the MHC contains a number of class I pseudogenes and gene fragments (17); however, all of the human class I sequences share close relationships indicating their common origin from a typical class I gene.

In this report, we describe a family of sequences[‡] in the human MHC which are highly divergent from all of the known MHC class I chains and have presumably been derived early in the evolution of mammalian class I genes. The *MICA* gene (*M*HC class *I* chain-related gene *A*) in this family was identified in a search of the HLA-B region for other expressed genes. This work was motivated by the still unexplained association of HLA-B27 with rheumatoid and inflammatory diseases. The uncommon organization of the *MICA* gene, its restricted expression, and the characteristic amino acid sequence of its product, all indicate that the putative MICA chain has evolved for a function that is related to but is quite distinct from that of typical MHC class I chains.[§]

MATERIALS AND METHODS

Isolation of *MICA* and *MICB* DNA Sequences. *MICA* and *MICB* cDNA clones were isolated from IMR90 human lung fibroblast (Clontech) and keratinocyte (18) libraries with a total insert probe prepared from cosmid M32A (19) by described procedures (19). The closely related *MICA* and *MICB* cDNA clones of 1.4 and 2.4 kb, respectively, were assigned to their corresponding genomic locations by blot hybridization with the previously cloned and mapped cosmids R9A, R5A, K7A, and M32A (19), by comparison of cDNA and genomic DNA sequences, and by sequencing across intron–exon junctions. Selected amino acid sequences were aligned by using the EUGENE program package (Baylor College of Medicine, Houston).

DNA and RNA Blot Hybridizations. Preparations of DNA from human B-cell lines and from yeast artificial chromosome (YAC) clones and of RNA from various human cell lines were according to standard protocols (20). All cell lines were from the American Type Culture Collection or the Tenth International Histocompatibility Workshop (21). Primate DNA samples were a gift from D. Watkins (University of Wisconsin). YAC clones Y2, Y3, Y17, Y18, Y19, Y22, and Y23 have been published (22, 23); Y24, Y25, Y36, Y37, Y38, Y67, Y54, and Y53 are from unpublished work (D.E.G.). Blot transfer and hybridization of DNA restriction fragments and RNA with [α-^{32}P]dCTP-labeled MICA cDNA were according to standard procedures (20). For Fig. 2, RNA samples of 20 μg per

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Abbreviations: MHC, major histocompatibility complex; YAC, yeast artificial chromosome.

[‡]The Human Gene Mapping Workshop symbols for these loci are *MICA*, *MCIB*, *MICC*, *MICD*, and *MICE*.

[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. L14848).

lane were fractionated in 1% agarose/2.2 M formaldehyde gels before blot transfer. Hybridization and washes were under stringent conditions (19). For Fig. 6*A*, the final wash was in 0.5× standard saline citrate (SSC; ref. 20) with 0.1% $NaDodSO_4$ at 65°C. For Figs. 5*A* and 6*B*, low-stringency conditions were employed. DNA blots were hybridized in 5× SSC containing 50% formamide, 50 mM Tris (pH 7.5), 10 mM $Na_4P_2O_7$, 5 mM EDTA, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 1% $NaDodSO_4$, 5% dextran sulfate, and sonicated salmon sperm DNA (150 μg/ml) for 16 hr at 37°C. Filters were washed in 2× SSC with 0.1% $NaDodSO_4$ at 37°C.

Cloning and Sequencing of *MICA* Allelic Variants. *MICA* fragments of 2250 bp including the α1, α2, and α3 domains were amplified by the polymerase chain reaction (PCR) from genomic DNA of the HLA homozygous typing cell lines EJ32B, MOU, PITOUT, and YAR (21). The oligonucleotides selected for PCR were derived from the 5′ end of the α1 exon (5′-GAGCCCCACAGTCTTCGT-3′) and the transmembrane sequence (3′-TGTAAGGTACAAAGACGAC-5′) of *MICA* and were tested with cosmid M32A DNA as a template. With this primer pair, no fragment was amplified from cosmid R9A DNA encoding MICB. PCR was carried out using GeneAmp reagents and AmpliTaq DNA polymerase (Perkin–Elmer/Cetus), 0.5 μg of template DNA, and 25 pmol of each primer. Samples were subjected to 40 cycles of PCR with intervals at 94°C (1 min), 55°C (1 min), and 72°C (2 min) and the products were directly cloned into the TA vector pCRII (Invitrogen). Sequences were obtained from double-stranded templates by using flanking SP6 and T7 primers (Promega) and several oligonucleotides derived from *MICA* intron sequences. These were 42 bp 3′ of the α1 exon (3′-GTCTTTTCAATCCCCGTC-5′), 39 and 40 bp 5′ and 3′ of the α2 exon, respectively (5′-TCACTTGGGTGGAAAGGTGAT-3′ and 3′-ACGATCTCAACGGAGTGGAGG-5′), and 40 bp 5′ of the α3 exon (5′-GTTCCTCTCCCCTCCTTAGA-3′). All of the *MICA* alleles were verified by sequencing of at least three clones derived from two independent PCR products.

RESULTS AND DISCUSSION

We have used previously cloned cosmids from the 170-kb interval between *HLA-B* and *BAT1* at the centromeric end of the MHC class I region (19) to screen various human cDNA

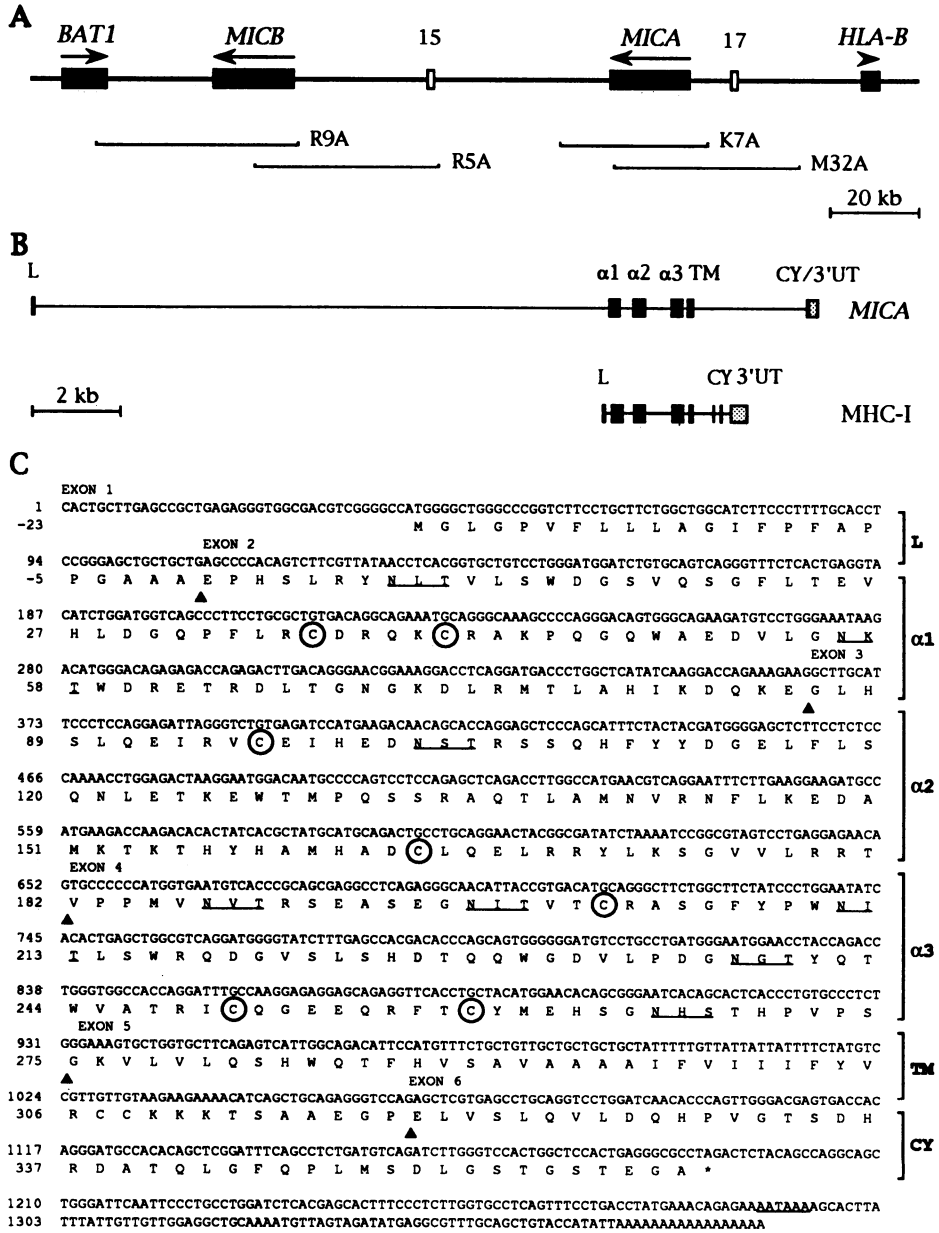

FIG. 1. (*A*) Localization of *MICA* and *MICB* in previously cloned cosmids. From a contiguous series, only the cosmids relevant here—R9A, R5A, K7A, and M32A—are shown; for a restriction map, see ref. 19. Black and shaded boxes on top line correspond to genes; open boxes labeled 15 and 17 indicate short class I-like gene fragments contained in *Xba* I and *Pst* I restriction fragments of 1.5 and 1.7 kb, respectively. Arrows show transcriptional orientations of genes. (*B*) Exon–intron organization of *MICA* and comparison with the canonical structure of MHC class I genes (1). Data were derived from cDNA and mapped cosmid DNA sequences. All splice junctions are between the first and second nucleotide of a codon. Closed and shaded boxes indicate exons. L, leader sequence; α1–α3, external domain sequences; TM, transmembrane segment; CY, cytoplasmic tail; 3′UT, 3′ untranslated sequence. (*C*) *MICA* cDNA sequence (upper lines with numbering at left) is of 1382 bp. The polyadenylylation signal is underlined. Lower lines show the translated amino acid sequence in the single-letter code. Numbering at left refers to the predicted mature protein, after cleavage of the putative signal peptide; the +1 position thus corresponds to the first amino acid in the α1 domain. Intron–exon junctions (see *B* and Fig. 3) are indicated by solid triangles and brackets at the right side. Cysteine residues are circled and potential sites for N-linked glycosylation (N-X-S/T) are underlined. See text for further explanations.

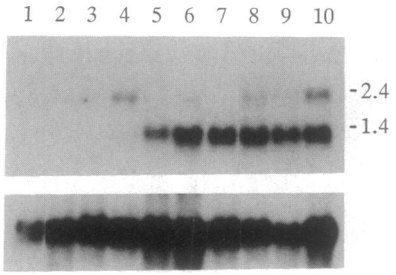

FIG. 2. (*Upper*) Expression of *MICA* in various cell lines. The RNA bands of almost 1.4 and about 2.4 kb are the *MICA* and *MICB* transcripts, respectively. Lane 1, Raji (B cell); lane 2, MOLT-4 (T cell); lane 3, HL-60 (promyelocyte); lane 4, U-937 (monocyte); lanes 5–10, fibroblast and epithelial cell lines (IMR-90, MRC-5, U-373, INT407, HeLa, and L-132, respectively). (*Lower*) Actin control.

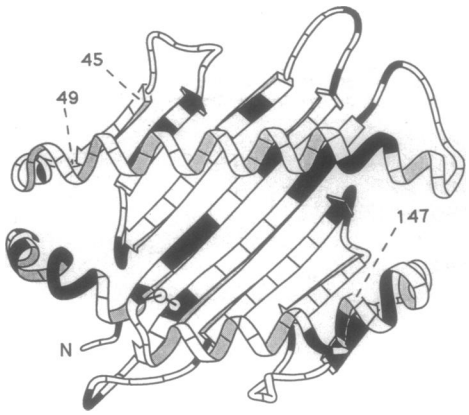

FIG. 4. Hypothetical placement of conserved MICA α1 and α2 residues on the ribbon diagram of HLA-A2 (adapted from ref. 30) based on the sequence alignment shown in Fig. 3. Major structural deviations of MICA include a deletion of amino acid positions 45–49 and an insertion of 6 amino acids at position 147.

libraries for homologous transcribed sequences. The *BAT1* gene flanking this region encodes a putative ATP-dependent nucleic acid helicase unrelated to any other MHC gene (unpublished data). *MICA* and *MICB* were identified by isolation of cDNA clones corresponding to two distant locations 40 and 110 kb from *HLA-B*, respectively (Fig. 1*A*). *MICA* and *MICB* are closely related, sharing 91% identity in their coding sequences; the *MICB* sequence will be recorded elsewhere.

The *MICA* sequence was derived from fibroblast and keratinocyte cDNA clones. The single long open reading frame of 1149 bp extends from a probable translation initiation codon (AUG) at nt 40 to a termination codon at nt 1189 (Fig. 1*C*). It encodes a polypeptide of 383 aa with a relative molecular mass of 43 kDa (including the putative leader peptide of 23 aa) (Fig. 1*C*). Both its sequence and predicted domain structure are similar to those of class I chains, including three external domains (α1, α2, and α3), a putative transmembrane segment, and a carboxyl-terminal cytoplasmic tail (1, 2). As in class I genes (1), these domains and a predicted leader peptide correspond to discrete exons in *MICA*; however, the leader sequence and the α1 exon are spaced by a large intron in *MICA*, and the cytoplasmic tail and 3′ untranslated sequences are fused in a single exon (Fig. 1 *B* and *C*). The organization of *MICA* is thus distinct from all known class I genes. Moreover, also contrasting the expression of typical class I genes, *MICA* RNA was neither detected in B- and T-cell lines (Fig. 2) nor upregulated by γ-interferon in HeLa cells (data not shown) but was equally abundant in various fibroblast and epithelial cell lines (Fig. 2). Interestingly, a similar expression pattern is also characteristic of several nonclassical mouse *Q* and *T* genes (24, 25).

Although MICA shares homology with many sequences in the immunoglobulin superfamily, it is most distinctively

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                α1
                        10        20        30        40        50        60        70        80        90
MICA            EPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRCDRQKCRAK.....PQGQWAEDVLGNKTWDRETRDLTGNGKDLRMTLAHIKDQKE (%hom)
HLA cons        GS--M--FY-SV-RP-RGEPR-IAVGYV-DTQ-V-F-SDAASPRMEPRA-WIEQEGPEYWDRETQIVKAQSQTDRES--TLRGYYNQSEA  (18)
HLA-A*0201      ||--|--|F-||-||-|||||-||||||-|||-|-|-|||||Q||||||-||||||||||G||RK|||H|||H|V--G||||||||||  (18)
HLA-B*2705      ||--|--|H-||-||-|||||-|-||||-||L-|-|-||||||E||||-|||||||||||||||C||KA|||||---||-R|||||||  (21)
HLA-G           ||--|--|SAA|-||-|||||-||M|||-|||-|-|-||S|C|||||||-|V|||||||EE||RNT||HA||||MN-Q||||||||||  (15)
H-2D^d          ||-----|V-A|-||-F||||YME||||-N|E-|-|-|||EN||Y||||R|||||||||E|||R-A|GNE|SF|V---|A-R|||||AG  (20)
H-2T22^b        ||-----||-A|-||-L|||W-|I|||||-|M|V--|S|KEET|||A...-|L|||-ADN|EQQ|R||-I|G|LSERN-M||VHF||K|MD  (19)
                      *
                α2
                       100       110       120       130       140             150       160       170       180
MICA            GLHSLQEIRVCEIHEDNST.RSSQHFYYDGELFLSQNLETKEWTMPQSSRAQTLAMNVRNFLKEDAMKTKTHYHAMHADCLQELRRYLKSGV.VLRRT (%hom)
HLA cons        -S-T--RMYG-DVGP-GRLL-GYHQYA---KDYIAL-EDLRS--AADTAAQI-QRKW......-A-RVAEQLRAYLEGT-VEW-----EN-KET-Q-A  (26)
HLA-A*0201      -|-|V-||||-|||S-W|F|-||||||---||||||K|||||--|||M|||T-KH||......-|-H|||||||||||-|||-----||-|||-|--  (25)
HLA-B*2705      -|-|--N|||-||||-||||-||||D|---||||||-|||S|--||||||||-||||......-|-||||||||||||E-|||-----||-|||-|-|  (26)
HLA-G           S|-|--W|I|-|L|S-||||-||E|||---|||-||-||||||--||||||||SK||C......-|-N||||R|||||||-|||-H---||-||M-|-|  (24)
H-2D^d          -|-|--W|A|-||ES-||||-||W|-|---C|||||-|||-T--|||M||||-R|||.....-Q-GA||RD||||||E-|||------|-NA|-L--  (30)
H-2T22^b        D|-|--WLQ|-||E|-RH|CLW|N|L|--S-|LPT|-|NPS|C-VGN-TVP..................HISQD|KSH-SDL-QK--|K-||R-L-S  (19)
                        Δ                           *                                 Δ     *
                α3
                      190       200       210       220       230       240       250       260       270
MICA            VPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWGDVLPDGNGTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPS (%hom)
HLA cons        D--KTH--HHPI-DHEA-LR-W-L----AE---T-QR--EDQ.TQDTELVETR-A-DR-F-K-A-VVVPS-----Y--HVQ-E-LPKPLTLRW  (35)
HLA-A*0201      |A-|||M-||AV-||||-||-|-|S---||---|-||--|||.||||||||||-|-|--|-|-|-|||||-Q---|--|||-|-|||||||||  (32)
HLA-B*2705      |--|||--||||-||||-||-|-|----||---|-||--|||.||||||||||-|-||-|-|-|-|||||-----|--|||-|-|||||||||  (35)
HLA-G           |--|||--|||VF|Y||-||-|-|----||-I-|-||--|||.|||V||||||-|-|--|-|-|-|||||-----|--|||-|-||E||M|||  (34)
H-2D^d          |--|A|--||RRP--DV-||-|-|----|D---|-|LN-|E-.||EM||||||-|-|--|-|-|S||||L-K--K|--||--|-||E||||||  (35)
H-2T22^b        |--|A|---||RP--DV-||-|-|----|D---|-|LN-|E-.|||M||||||-|-|--|-|-|-||||L-K--S|--||Y-|-||E||I|||  (36)
                              Δ                                                       Δ
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FIG. 3. Distant homology of the MICA amino acid sequence with human and mouse MHC class I chains. In the computer-assisted multiple alignment, the α1, α2, and α3 domains of various selected human sequences (HLA consensus, HLA-A*0201, HLA-B*2705, and HLA-G; ref. 2) and mouse sequences (H-2T22^b, H-2D^b; refs. 10 and 28) are separately compared with the corresponding MICA sequences. Gaps were introduced to maximize homology, which is indicated as percent identity at right. Dashes show identical residues. Vertical lines in the lower five class I sequences identify residues shared with the human class I HLA consensus sequence (second line) (2). This dual representation emphasizes the distinct derivation of MICA. Open triangles below the α2 and α3 sequences indicate the highly conserved cysteine residues, which are at amino acid positions 101, 164, 203, and 259 in typical class I chains (2). Stars below the α1 and α2 sequences identify conserved residues involved in the tight binding of peptides by class I molecules (29).

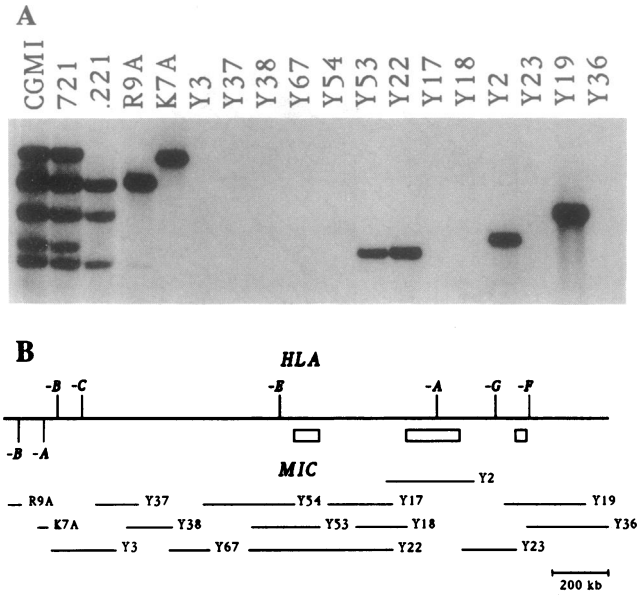

FIG. 5. Identification and mapping of additional sequences related to *MICA* in the MHC. (*A*) The *MICA* cDNA hybridized to five *Hin*dIII restriction fragments in genomic DNA from the CGMI and 721 human B-cell lines under conditions of low stringency (see *Materials and Methods*). The upper two bands correspond to *MICB* and *MICA*, which are contained in the cosmids R9A and K7A, respectively (see Fig. 1*A*). *MICA* and another band were missing in DNA from mutant 721.221, in which *HLA-A* and *HLA-B* are deleted (34). The three novel bands were matched by specifically hybridizing fragments in overlapping YACs (lanes Y3–Y36) spanning the entire MHC class I region. (*B*) Approximate locations of *MIC*-like bands, MICC, MICD, and MICE near *HLA-E*, *-A*, and *-F*, respectively, are indicated by open boxes below the map of the class I region (13, 22, 23). Lines below the map show the locations of cosmids and YACs.

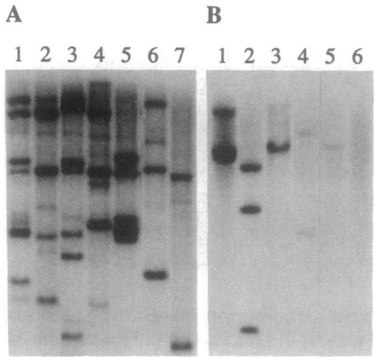

FIG. 6. Evolutionary conservation of *MICA*. (*A*) *MICA*-related bands were detected in *Asp* 718/*Xba* I-digested DNA samples from various primates under stringent conditions: human, chimpanzee, orangutan, baboon, gibbon, marmoset, and tamarin (lanes 1–7, respectively). (*B*) At low stringency (see *Materials and Methods*), specific bands were also detected in *Hin*dIII-digested DNA from goat, pig, cow, dog, and hamster (lanes 1–5), but not in mouse DNA (lane 6).

related to mammalian MHC class I chains and not to the class I-like CD1 (26) and Fc receptor (27) sequences encoded outside the MHC. MICA is about equidistant from various human and mouse class I chains, with 15–21% and 19–30% amino acid sequence identity in the α1 and α2 domains, respectively, and with 32–36% identity in the immunoglobulin-like α3 domain (Fig. 3). These degrees of similarity are substantially lower than those found among the MHC class I chains in any species. For example, the mouse nonclassical H-2T22 and H-2M3 chains share an average of 60% and 67% identity in their α1–α3 domains with the H-2K class I chain, respectively (10, 11). All of the human MHC class I chains have diverged to a lesser extent. These relationships indicate an independent derivation of *MICA*, which must have been adapted to a modified function, presumably early in the evolution of mammalian MHC class I genes.

Comparison of MICA with other class I chains shows that most of the matching residues are common to all of the aligned sequences (Figs. 3 and 4). Some of these are universally conserved in MHC class I chains throughout vertebrate evolution, which are extraordinarily diverse (31, 32). Only a few residues, 3 in α1 (His-3, Thr-10, and Asp-29), 4 in α2 (Gln-96, Cys-101, Gly-120, and Cys-164), and 11 in α3 (Pro-185, Phe-208, Tyr-209, Pro-210, Pro-235, Asp-238, Cys-259, Val-261, His-263, Leu-266, and Pro-269) have been found to be invariant in typical class I sequences from all vertebrate classes and can therefore be considered most critical for the structure of class I molecules (32). Their positions are at intra- and interdomain contacts and in or between β-strands, but not in the α-helices involved in peptide binding and T-cell recognition (30, 32). Remarkably, except for four substitutions in the α3 domain (positions 238, 261, 266, and 269), all of these residues are present in MICA, including the two pairs of cysteines in the α2 and α3 domains, which form intradomain disulfide loops (Figs. 3 and 4). Thus, it is likely that the putative MICA chain folds similarly to class I chains, although it is highly diverged. Further characteristic of MICA are its unique transmembrane and cytoplasmic tail sequences, 3 extra cysteine residues in the α1 and α3 domains, and several potential N-linked glycosylation sites (Fig. 1*C*). Specific residues implicated in the binding of the CD8 coreceptor to class I molecules (33) are absent from MICA. But

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1  EPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRCDRQKCRAKPQGQWAEDVLGNKTWDRETRDLTGNGKDLRMTLAHIKDQKE
2  .............G.........A.....................................*.....................
3  ......................*A.....................................*.....................
4  ......................*A............Y........................*.....................
5  ......................*A............Y........................*.....................

1  GLHSLQEIRVCEIHEDNSTRSSQHFYYDGELFLSQNLETKEWTMPQSSRAQTLAMNVRNFLKEDAMKTKTHYHAMHADCLQELRRYLKSGVVLRRT
2  ....................................E.....................................................
3  ..................................V..E...V.....................................E.S.....R
4  ..................................V..E...V.....................................E.S.....R
5  ....................................E...V.....................................................

1  VPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWGDVLPDGNGTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPS
2  ....................................................................................
3  ................*.....*S...R....T.....................................................G......
4  ................*.....*S...R....T.....................................................
5  ......................*S...R....T.....................................R.....................
```

FIG. 7. Polymorphism of MICA. Amino acid sequences 1–5 represent five *MICA* alleles encoding variant residues in the α1 (*Top*), α2 (*Middle*), and α3 (*Bottom*) domains. Alleles 1 and 5 correspond to the IMR-90 and keratinocyte cDNA sequences, respectively. Alleles 1–4 were identified in the HLA homozygous typing cell lines EJ32B (allele 1), YAR (allele 2), PITOUT (alleles 3, 4), and MOU (allele 4). Stars indicate silent nucleotide substitutions.

some of the amino acid side chains known to interact with the termini of the short peptides bound by typical class I molecules (29) are preserved (Figs. 3 and 4). Of 4 tyrosine side chains (positions 7, 59, 159, and 171) forming hydrogen bonds with the amino-terminal residue of bound peptide, Tyr-7 and Tyr-171 are present in MICA. In the putative carboxyl-terminal binding pocket, Thr-143 is conserved, but Tyr-84, Lys-146, and Trp-147 are variously substituted. Although the characteristic binding of the peptide terminus by typical class I molecules may thus be altered in MICA, these data suggest that the putative MICA chain may have the capacity to associate with peptide ligands, but it is also possible that a different, nonpeptide moiety is bound.

As the class I gene family has evolved by sequential duplications, we investigated whether additional sequences related to *MICA* and *MICB* are encoded in the MHC. Under conditions of low stringency, the MICA cDNA hybridized to three fragments in total genomic DNA which were not accounted for by *MICA* and *MICB*. By using a series of overlapping YAC clones (refs. 22 and 23; D.E.G., unpublished data), these fragments were mapped to distinct locations in the vicinities of the *HLA-E*, *-A*, and *-F* genes (Fig. 5). These results demonstrate the existence of a gene family and indicate that a primordial *MIC* gene originated from a prototypic class I gene before the evolution of the class I gene family. This is further supported by the conservation of MIC-related sequences not only in primates (Fig. 6*A*) but also in various phylogenetically distant mammalian species (Fig. 6*B*). These characteristics clearly distinguish the *MIC* gene family from the nonclassical mouse *H-2Q*, *-T*, and *-M* genes, which are organized in gene clusters that are segregated from the classical *H-2K*, *-D*, and *-L* genes (5–8) and exist only in the mouse and perhaps some closely related rodents. However, it is peculiar that MICA and the $T22^b$ chain, which can be recognized by $\gamma\delta$ T cells (10), share a similar gap in the $\alpha 1$ domain (Fig. 3); the missing residues are normally associated with the "45" pocket (Fig. 4), which binds the P2 peptide side chain in some but not all typical class I molecules (29, 35). This common feature could be the result of an independent adaptation to a similar function, as no MIC-like sequences were detected in mouse DNA (Fig. 6*B*), either because of their divergence or possibly because of loss.

Taken together, these results define a second lineage of evolutionarily conserved MHC class I genes. This relationship implies a specialized, conceivably ancient role of *MIC* gene products in some aspect of antigen presentation and T-cell recognition. This is indirectly supported by preliminary data indicating that *MICA* is polymorphic. A comparison of *MICA* sequences derived from two different cDNA libraries and four HLA homozygous typing cell lines revealed five different alleles with altogether 18 nucleotide changes in the $\alpha 1$–$\alpha 3$ domains, of which 14 result in amino acid substitutions (Fig. 7). These data indicate a potential relevance of *MICA* to autoimmunity and transplantation, but their thorough interpretation will require exact knowledge of the function of the MICA protein.

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